NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 435

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 4,4′-THIOBIS(6-*t*-BUTYL-*m*-CRESOL)

(CAS NO. 96-69-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from NTP Central Data Management, NIEHS, P.O. Box 12233, MD A0-01, Research Triangle Park, NC 27709 (919-541-1371).

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

4,4′-THIOBIS(6-*t*-BUTYL-*m*-CRESOL)

(CAS NO. 96-69-5)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

December 1994

NTP TR 435

NIH Publication No. 95-3166

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

C.J. Alden, Ph.D.
G.A. Boorman, D.V.M., Ph.D.
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
J.D. Cirvello, B.S.
S.L. Eustis, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
G.N. Rao, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
B.A. Schwetz, D.V.M., Ph.D.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Battelle Columbus Laboratories

Conducted studies, evaluated pathology findings

P.J. Kurtz, Ph.D., Principal Investigator
M. Grabaskas
M.R. Hejtmancik, Ph.D.
J.D. Toft II, D.V.M., M.S.

Experimental Pathology Laboratories, Inc.

Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
B.F. Hamilton, D.V.M., Ph.D.

Dynamac Corporation

Prepared pathology audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group

Evaluated slides, prepared pathology report on rats (10 September 1991)

R.M. Kovatch, D.V.M., Chair
Pathology Associates, Inc.
K.M. Ayers, D.V.M.
Burroughs Wellcome Laboratories
S. Ching, D.V.M., Ph.D.
Merck Research Laboratories
J. Cullen, V.M.D., Ph.D.
North Carolina State University
M.R. Elwell, D.V.M., Ph.D.
National Toxicology Program
B.F. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories
M.P. Jokinen, D.V.M.
National Toxicology Program

Evaluated slides, prepared pathology report on mice (26 September 1991)

T.M. Monticello, D.V.M., Ph.D., Chair
Pathology Associates, Inc.
B.F. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories
R.H. Herbert, D.V.M., Ph.D.
National Toxicology Program
M.P. Jokinen, D.V.M.
National Toxicology Program
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program

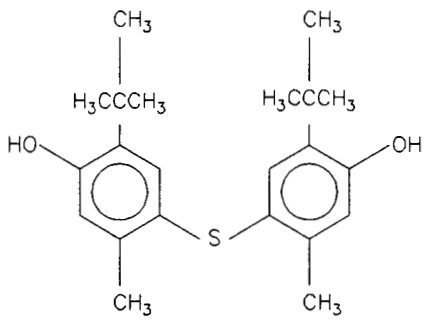
Biotechnical Services, Inc.

Prepared Technical Report

D.D. Lambright, Ph.D., Principal Investigator
G. Gordon, M.A.
P.S. Keightley, B.A.
T.A. King-Hunter, B.S.
H.A. Lindsay, B.A.

CONTENTS

ABSTRACT



4,4′-THIOBIS(6-*t*-BUTYL-*m*-CRESOL)

CAS No. 96-69-5

Chemical Formula: $C_{22}H_{30}SO_2$ Molecular Weight: 358.52

Synonyms: 4,4′-Thiobis(6-*t*-butyl-3-cresol); bis(3-*t*-butyl-4-hydroxy-6-methylphenyl)sulfide
Trade names: Santonox; Santowhite Crystals; Sumilizer; Thioalkofen; Yoshinox

4,4′-Thiobis(6-*t*-butyl-*m*-cresol) (TBBC) is used in the rubber and plastics industries as an antioxidant. TBBC is also used as a stabilizer in polyethylene and polyolefin packaging materials for foodstuffs. Toxicology and carcinogenesis studies were conducted by administering TBBC (99% pure) in feed to groups of male and female F344/N rats and B6C3F$_1$ mice for 15 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and cultured Chinese hamster ovary cells.

15-DAY STUDY IN RATS

Groups of 10 male and 10 female F344/N rats were fed diets containing 0, 1,000, 2,500, 5,000, 10,000 or 25,000 ppm TBBC for 15 days. Rats given to 1,000, 2,500, 5,000, or 10,000 ppm received approximate doses of 95, 235, 335, or 365 mg TBBC per kilogram body weight per day (males) or 85, 220, 325, or 270 mg/kg per day (females). Approximate doses for rats receiving 25,000 ppm could not be calculated due to early deaths. All 25,000 ppm rats and three male and four female 10,000 ppm rats died. Surviving rats in the 10,000 ppm groups had a significant weight loss and the final mean body weights of 5,000 and 10,000 ppm male and female rats were significantly lower than those of the controls. Male and female rats exposed to 5,000, 10,000, or 25,000 ppm TBBC consumed markedly less feed than the controls.

Diarrhea occurred in 5,000, 10,000, and 25,000 ppm males and females. The principal lesions attributed to the administration of TBBC were renal papillary and tubule necroses which occurred in 10,000 ppm rats. Focal necrosis or erosions of the glandular stomach also occurred in some 10,000 ppm rats. Changes observed in the thymus and spleen were attributed to debilitation or stress; bone marrow depletion was attributed to nutrient deficiency accompanying weight loss.

15-DAY STUDY IN MICE

Groups of 10 male and 10 female B6C3F$_1$ mice were fed diets containing 0, 1,000, 2,500, 5,000, 10,000, or 25,000 ppm TBBC for 15 days. Mice given 1,000, 2,500, or 5,000 ppm received approximate doses of 285, 585, or 475 mg TBBC per kilogram body weight per day (males) or 360, 950, or 1,030 mg/kg per day (females). Approximate doses for mice given 10,000 or 25,000 ppm could not be calculated due to early deaths. All 10,000 and 25,000 ppm mice died, as did eight males and eight females given 5,000 ppm. A

significant weight loss occurred in surviving 5,000 ppm males and females and the final mean body weights of 2,500 ppm females and 5,000 ppm males and females were significantly lower than those of the controls. Feed consumption by mice given 5,000, 10,000, or 25,000 ppm was markedly reduced. Diarrhea occurred in all 25,000 ppm mice and in most male and female mice given 5,000 or 10,000 ppm. Renal tubule necrosis occurred in eight males and three females in the 5,000 ppm groups. Lymphocytic depletion of lymphoid tissues in many 5,000 ppm males and females was attributed to debilitation and stress or to nutrient deficiency accompanying weight loss.

13-Week Study in Rats

Groups of 10 male and 10 female F344/N rats were fed diets containing 0, 250, 500, 1,000, 2,500, or 5,000 ppm TBBC for 13 weeks. These exposure levels delivered approximate doses of 15, 30, 60, 165, or 315 mg TBBC per kilogram body weight per day (males) or 15, 35, 70, 170, or 325 mg/kg per day (females). All rats survived to the end of the study. The final mean body weight of 5,000 ppm males was 40% lower than that of the controls; the final mean body weight of 5,000 ppm females was 27% lower than that of the controls. Feed consumption by male and female rats exposed to 5,000 ppm TBBC was markedly lower than that by the controls throughout the study. The absolute and relative liver weights of 5,000 ppm females were significantly greater than those of the controls.

Serum alkaline phosphatase (ALP) levels were significantly higher in 2,500 and 5,000 ppm males and slightly higher in 5,000 ppm females. Serum alanine aminotransferase levels were significantly higher in 2,500 and 5,000 ppm males and females. Hematocrit and hemoglobin concentrations and mean erythrocyte volume (MCV) values were significantly lower in 1,000, 2,500, and 5,000 ppm males than in controls; MCV values were also significantly lower in 5,000 ppm females. A dose-related significant increase in forelimb and hindlimb grip strength was observed in exposed male and female rats.

Histopathologic findings in the liver of 2,500 and 5,000 ppm males and females included hypertrophy of Kupffer cells, bile duct hyperplasia, and individual cell necrosis of hepatocytes; centrilobular hepatocyte hypertrophy also occurred in males and females exposed to 5,000 ppm TBBC. Macrophages were increased in size and number in the mesenteric lymph nodes of males and females exposed to 5,000 ppm, and to a lesser extent in 2,500 ppm male and female rats. Pigmentation and degeneration of the renal cortical tubule epithelial cells was also present in males and females in the 2,500 and 5,000 ppm groups; cortical tubule necrosis occurred in 5,000 ppm males and females.

13-Week Study in Mice

Groups of up to 10 male and 10 female B6C3F$_1$ mice were fed diets containing 0, 100, 250, 500, 1,000, or 2,500 ppm TBBC for 13 weeks. These exposure levels delivered approximate doses of 15, 30, 65, 145, or 345 mg TBBC per kilogram body weight per day (males) or 10, 35, 60, 165, or 340 mg/kg per day (females). All mice survived to the end of the study. The final mean body weights of 2,500 ppm males and of 500, 1,000, or 2,500 ppm females were significantly lower than those of the controls. Feed consumption by 2,500 ppm males averaged 24% lower than that by controls through week 3 and was similar to that by controls for the remainder of the study. Feed consumption by females receiving 2,500 ppm averaged 27% less than that by the controls during most of the study. The absolute and relative liver weights of males and females exposed to 2,500 ppm TBBC were slightly but significantly greater than those of the controls. Males exposed to 500, 1,000, or 2,500 ppm and females exposed to 2,500 ppm had significantly increased absolute and relative spleen weights. No clinical findings in mice were considered chemical related.

Hematocrit concentrations and erythrocyte counts of males receiving 1,000 or 2,500 ppm were significantly less than those of the controls; hemoglobin concentration in males receiving 2,500 ppm was significantly less and mean erythrocyte volume was significantly less in males receiving 2,500 ppm. Females in the 1,000 and 2,500 ppm groups had significantly decreased hematocrit concentrations and erythrocyte counts; 2,500 ppm females also had significantly decreased hemoglobin concentrations and mean erythrocyte volumes.

Kupffer cell hypertrophy, bile duct hyperplasia, and an increase in size and number of macrophages in mesenteric lymph nodes were present in 2,500 ppm male and female mice.

2-YEAR STUDY IN RATS

Doses selected for the 2-year study of TBBC were based on the lower body weights and liver and kidney toxicity observed at 5,000 ppm in the 13-week study.

Groups of 115 male and 75 female F344/N rats were fed diets containing 0, 500, 1,000, or 2,500 ppm TBBC for 2 years. Based on average daily feed consumption, these exposure levels resulted in a daily ingestion of TBBC of approximately 20, 40, or 100 mg/kg body weight for males and 20, 45, or 120 mg/kg body weight for females. Hematology, clinical chemistry, and urinalysis evaluations were performed on 15 male and 15 female rats from each group at 3, 9, and 15 months. Also at 15 months, an additional 10 male and 10 female rats from each group were evaluated for histopathology, hematology, and clinical chemistry. Forty male rats per group were evaluated for neurotoxic effects.

Survival, Body Weights, Feed Consumption, and Clinical Findings

Two-year survival rates and mean body weights of exposed male and female rats were generally similar to those of the controls. The mean body weights of 2,500 ppm male rats were slightly lower than those of the controls throughout the study. At week 65, the mean body weight of 2,500 ppm females was 14% lower than that of the controls, but the final mean body weight of this group was 6% lower than that of the control group. Feed consumption, behavior, and general health and appearance of exposed male and female rats were similar to those of the controls.

Hematology and Clinical Chemistry

Results of the hematology evaluation were not uniformly consistent at 3, 9, and 15 months in one set of rats, nor were they consistent between the two sets of rats evaluated at 15 months. Slight but significant decreases in hematocrit levels, hemoglobin concentrations, and erythrocyte counts were observed in the 1,000 and 2,500 ppm groups in one set of males at 15 months. Similar significant decreases in hematocrit level and hemoglobin concentration occurred in 2,500 ppm females at 9 months. Mean erythrocyte hemoglobin and mean erythrocyte hemoglobin concentration of 2,500 ppm females were also significantly lower than those of controls at 9 months and in both sets of female rats evaluated at 15 months. Platelet counts of 2,500 ppm male and female rats were slightly but significantly higher than those of controls at 3 and 9 months. Platelet counts were also slightly but significantly increased in 2,500 ppm males of one set evaluated at 15 months, and in 2,500 ppm females of the second set evaluated at 15 months.

Serum activities of alkaline phosphatase, alanine aminotransferase, and sorbitol dehydrogenase in 2,500 ppm males were significantly greater than those in the controls at 3, 9, and 15 months. Alkaline phosphatase activities in both sets of 1,000 ppm males evaluated at 15 months were also significantly greater than those of controls. Serum activities of alanine aminotransferase and sorbitol dehydrogenase in 2,500 ppm females were also significantly greater than those in controls at 3, 9, and 15 months.

Neurotoxicity Findings

There were no significant inhibitory effects of TBBC on motor nerve excitability or conduction, neuromuscular transmission, or muscle contractility. There were no microscopic lesions in the sciatic nerve, quadriceps muscle, or teased nerve preparations of sciatic nerve that could be attributed to TBBC administration.

Pathology Findings

At the 15-month interim evaluation, the absolute and relative liver weights of 2,500 ppm female rats were significantly greater than those of controls; at 15 months and at the end of the study, the incidences of Kupffer cell hypertrophy, hepatocyte cytoplasmic vacuolization, and mixed cell foci were also significantly increased. At the end of the study, the incidence of hepatocellular fatty change was significantly increased in 2,500 ppm females. The incidence of Kupffer cell hypertrophy was significantly increased in 2,500 ppm males at 15 months and at 2 years; the incidence of cytoplasmic vacuolization was significantly increased in all exposed males at 15 months but only moderately increased in 1,000 and 2,500 ppm males at 2 years; the incidence of basophilic foci was significantly increased in 2,500 ppm males at 15 months and the incidence of mixed cell foci was significantly increased in 1,000 and 2,500 ppm male rats at 2 years. The incidences of hepatocellular adenoma or carcinoma (combined)

in exposed male rats were not significantly greater than that in the controls (0 ppm, 1/50; 500 ppm, 3/50; 1,000 ppm, 3/50; 2,500 ppm, 5/49), were within the historical control range, and were not considered chemical related. The severity of nephropathy was significantly increased in 2,500 ppm female rats.

There was a significant negative trend in the incidence of mammary gland fibroadenoma, adenoma, or carcinoma (combined) in female rats (32/50, 24/50, 11/50, 16/50), and the incidences of fibroadenoma in 1,000 and 2,500 ppm females were significantly less than that of the controls.

2-Year Study in Mice

Because of the reduction in body weights, the increase in liver and spleen weights, and the accompanying histopathologic changes in the liver of 2,500 ppm male and female mice in the 13-week study, the doses selected for the 2-year study were 250, 500, and 1,000 ppm.

Groups of 80 male and 80 female mice were fed diets containing 0, 250, 500, or 1,000 ppm TBBC for 2 years. Based on average daily feed consumption, these exposure levels resulted in the daily ingestion of approximately 30, 60, or 145 mg TBBC/kg body weight for males and 45, 110, or 255 mg TBBC/kg body weight for females. Nine or 10 animals from each exposure group were evaluated at 3, 9, and 15 months.

Survival, Body Weights, Feed Consumption, and Clinical Findings

Two-year survival rates of exposed male and female mice were similar to those of the controls. The final mean body weights of male and female mice exposed to 1,000 ppm were 8% and 18% lower than those of the controls, respectively. The final mean body weights of females exposed to 250 or 500 ppm were 8% to 9% lower than that of the controls. Feed consumption by exposed males was similar to that by controls, and there were no clinical findings attributed to TBBC administration.

Hematology and Clinical Chemistry

Hematocrit level, hemoglobin concentration, and erythrocyte count in 1,000 ppm male mice were significantly lower than those in controls at the 15-month interim evaluation. Serum alkaline phosphatase activities in 1,000 ppm males were slightly but significantly greater than those in controls at 3 and 9 months, as was the serum alkaline phosphatase activity in 1,000 ppm females at 9 months. Serum levels of total bilirubin in all exposed groups of males were significantly greater than those in controls at 9 and 15 months.

Pathology Findings

In the liver of male mice, negative trends in the incidences of fatty change, clear cell foci, and adenoma or carcinoma combined occurred at the end of the 2-year study. There were no compound-related increased incidences of neoplasms or nonneoplastic lesions in mice receiving TBBC for 2 years. A negative trend in the incidence of fatty change in the liver of male mice also occurred at 15 months.

Genetic Toxicology

4,4′-Thiobis(6-*t*-butyl-*m*-cresol) was not mutagenic in *Salmonella typhimurium* strains TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation (S9). Sister chromatid exchanges were induced in cultured Chinese hamster ovary cells treated with TBBC, with and without S9, but no increases in chromosomal aberrations were noted in cultured Chinese hamster ovary cells after treatment with TBBC.

Conclusions

Under the conditions of these 2-year feed studies, there was *no evidence of carcinogenic activity** of 4,4′-thiobis(6-*t*-butyl-*m*-cresol) in male or female F344/N rats administered 500, 1,000, or 2,500 ppm or in male or female B6C3F$_1$ mice administered 250, 500, or 1,000 ppm.

Nonneoplastic lesions associated with exposure to TBBC included: Kupffer cell hypertrophy, cytoplasmic vacuolization, and mixed cell foci in the liver of male and female rats, fatty change in the liver of female rats, and an increase in the severity of nephropathy in the kidney of female rats. In addition, decreased incidences of fibroadenoma, adenoma, or carcinoma (combined) were observed in the mammary gland of female rats. Decreases also occurred in the incidences of fatty change, clear cell foci, and adenoma or carcinoma (combined) in the liver of male mice.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 4,4′-Thiobis(6-*t*-Butyl-*m*-Cresol)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 500, 1,000, or 2,500 ppm in feed (approximately 20, 40, or 100 mg/kg/day)	0, 500, 1,000, or 2,500 ppm in feed (approximately 20, 45, or 120 mg/kg/day)	0, 250, 500, or 1,000 ppm in feed (approximately 30, 60, or 145 mg/kg/day)	0, 250, 500, or 1,000 ppm in feed (approximately 45, 110, or 255 mg/kg/day)
Body weights	Exposed groups lower than controls	2,500 ppm group lower than controls	1,000 ppm group lower than controls	Exposed groups lower than controls
2-Year survival rates	18/50, 28/50, 22/50, 18/50	34/50, 31/50, 32/50, 28/50	42/50, 42/50, 49/50, 45/50	40/51, 38/50, 36/50, 35/50
Nonneoplastic effects	Liver: Kupffer cell hypertrophy: 2/50, 3/50, 2/50, 31/49; cytoplasmic vacuolization: 13/50, 11/50, 19/50, 18/49; mixed cell foci: 6/50, 14/50, 18/50, 15/49	Liver: Kupffer cell hypertrophy: 11/50, 10/50, 9/50, 42/50; cytoplasmic vacuolization: 12/50, 10/50, 20/50, 34/50; fatty change: 9/50, 8/50, 15/50, 19/50; mixed cell foci: 5/50, 4/50, 14/50, 34/50 Kidney: nephropathy severity (1.4, 1.4, 1.6, 2.3)	None	None
Neoplastic effects	None	None	None	None
Other findings	None	Mammary gland: fibroadenoma, adenoma, or carcinoma (combined): 32/50, 24/50, 11/50, 16/50	Liver: fatty change: 19/50, 17/50, 5/50, 6/50; clear cell foci: 6/50, 5/50, 2/50, 0/50; adenoma or carcinoma (combined): 25/50, 30/50, 27/50, 16/50	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence

Genetic toxicology

Salmonella typhimurium gene mutation: Negative in strains TA98, TA100, TA1535, and TA1537 with and without S9

Chinese hamster ovary cells *in vitro*

Sister chromatid exchanges: Positive with and without S9

Chromosomal aberrations: Negative with and without S9

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on 4,4′-thiobis(6-*t*-butyl-*m*-cresol) on June 22, 1993, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

Curtis D. Klaassen, Ph.D., Chair
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Paul T. Bailey, Ph.D.
Environmental and Health Sciences Laboratory
Mobil Oil Corporation
Princeton, NJ

Louis S. Beliczky, M.S., M.P.H., Principal Reviewer
Department of Industrial Hygiene
United Rubber Workers International Union
Akron, OH

Arnold L. Brown, M.D.
University of Wisconsin Medical School
Madison, WI

Kowetha A. Davidson, Ph.D.
Health and Safety Research Division
Oak Ridge National Laboratory
Oak Ridge, TN

Harold Davis, D.V.M., Ph.D.
Medical Research Division
American Cyanamid
Pearl River, NY

Daniel S. Longnecker, M.D.*
Department of Pathology
Dartmouth Medical School
Lebanon, NH

Louise Ryan, Ph.D.
Division of Biostatistics
Harvard School of Public Health and
Dana-Farber Cancer Institute
Boston, MA

Ellen K. Silbergeld, Ph.D.*
University of Maryland Medical School
Baltimore, MD

Robert E. Taylor, M.D., Ph.D.
Department of Pharmacology
Howard University College of Medicine
Washington, D.C.

Matthew J. van Zwieten, D.V.M., Ph.D.
Department of Safety Assessment
Merck Research Laboratories
West Point, PA

Jerrold M. Ward, D.V.M., Ph.D., Principal Reviewer
National Cancer Institute
Frederick, MD

Lauren Zeise, Ph.D., Principal Reviewer
Reproductive and Cancer Hazard Assessment Section
California Environmental Protection Agency
Berkeley, CA

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 22, 1993, the draft Technical Report on the toxicology and carcinogenesis studies of 4,4′-thiobis(6-*t*-butyl-*m*-cresol) (TBBC) received public review by the National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Mr. J.D. Cirvello, NIEHS, introduced the toxicology and carcinogenesis studies of TBBC by discussing the uses of the chemical and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related nonneoplastic lesions in rats and mice. The proposed conclusions were *no evidence of carcinogenic activity* of 4,4′-thiobis(6-*t*-butyl-*m*-cresol) in male or female F344/N rats or male or female B6C3F$_1$ mice.

Mr. Beliczky, a principal reviewer, agreed with the proposed conclusions. He asked if the literature had been reviewed as most of the references were from the 1950's. Mr. Cirvello said a literature search had been done in 1992. Mr. Beliczky questioned the reference to the NIOSH Permissible Exposure Limit because the levels that were mentioned as either total dust or respirable dust are generally referred to as nuisance dust, those dusts which are physiologically inactive or inert. He did not think one could call TBBC inert or physiologically inactive. He commented that the nomination for review by the NTP was referenced to a 1978 study at Harvard and wanted to note that this epidemiological study had been funded by the United Rubber Worker's Joint Occupational Health Program.

Dr. Zeise, the second principal reviewer, agreed in principle with the proposed conclusions. She pointed out that, while the liver in male rats is clearly a target organ for toxicity, the data are unclear as to whether or not the liver is a target organ for carcinogenicity. She said the incidence of hepatocellular adenoma would be statistically significant if the historical control incidence at the study laboratory were used instead of the concurrent controls. She said there should be consideration given to changing the conclusion in male rats to "equivocal evidence of carcinogenic activity." Mr. Cirvello commented that if one looks at the overall historical control database, there were three studies from other laboratories with control values as high as those recorded in male rats in the high-dose group in the present study.

Dr. Ward, the third principal reviewer, agreed in principle with the proposed conclusions. He said it should be noted that the degree of nephropathy was increased in female rats and there should be a statement that male rats may have been able to tolerate a slightly higher dose. Mr. Cirvello said a statement about the nephropathy should have been included. He said that toxicity and reduction in body weight gain in the prechronic and 2-year studies indicated that the high dose was correct in male rats. Dr. Ward agreed with Dr. Zeise as to the uncertain significance of the liver neoplasms in male rats. Since mixed cell foci were increased more in exposed animals, Dr. Ward said it would be useful to have a morphologic description and an assessment as to whether they are preneoplastic lesions. Dr. S.L. Eustis, NIEHS, said a description would be added to the report, but it was difficult to say whether the foci were preneoplastic. There was no atypia reported, a finding often found in foci induced by hepatocarcinogens.

Mr. Beliczky moved that the Technical Report on 4,4′-thiobis(6-*t*-butyl-*m*-cresol) be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, *no evidence of carcinogenic activity*. Dr. Bailey seconded the motion, which was accepted unanimously with ten votes.